EXPERIMENTAL EMBRYOLOGY.

By E. A. Andrews.

(*Continued from May number, p. 382.*)

Schultze[1] holds that the black pole becomes the dorsal region on which the medullary folds are formed, and that the blastopore arises and remains near the tail end of the animal, at the highest part of the white yolk, when the egg is, as is normal he says, inclined about 45°. There is, however, a rotation of the egg about a horizontal axis at right angles to the plane of symmetry, a rotation that carries blastopore downward 80° Yet this is compensated for by a reverse rotation upward of 90°, so that there is little absolute change after the blastopore is closed; the ingrowth of entoderm during gastrulation being, he surmises, the cause of these revolutions, since the egg is thereby overbalanced, first one way then the other.

After this digression beyond the limits of experimental embryology into the hazy ground of unverified hypotheses we may turn attention to a work rich in experimentation, the only French contribution that we are acquainted with, the very suggestive work upon the ascidian egg by Chabry,[2] whose paper appears not to have met with the appreciation it deserves.

Having made a very careful study of the cleavage phenomena in normal eggs of *Ascidia aspera* in the summer seasons of 1884 and 1886, the author was in a position to appreciate the remarkable abnormalities sometimes occurring in the development of this ascidian. As these abnormalities to some extent correspond with the results of artificial treatment of the eggs, some account of them cannot be passed over here,

[1]O. Schultze, Ueber Axenbestimmung des Froshembryo. Biol. Centb., vii, 1888, pp. 577–588.

[2]Contribution a l'embryologie normale et tératologique des ascidies simples. Jour. de l'Anatomie, 1887, pp. 167–313, plates 18–22.

especially as they furnish in themselves interesting facts bearing upon our interpretation of embryological phenomena.

Without apparent cause, unless, as the author inclines to believe, old age of the adults is here concerned, all the ascidians obtained late in one season gave rise to abnormal eggs, few amid the entire number developing in the normal way for any length of time. These natural monsters or deficient eggs can be explained only on the assumption that the parent organism made them imperfect from the start, or at least furnished abnormal conditions of environment for them before they were laid, as they may develop in the same aquaria with other eggs that follow a typical series of changes without any abnormalities. Moreover the eggs from one adult often show some common defect or tendency to be abnormal in certain lines, though there is great individual difference between even these eggs.

These abnormalities of unknown or natural origin are classified under the following seven heads: 1st, change from the normal position of the cleavage planes; 2d, retarded cleavage; 3d, cleavage confined to the nuclei; 4th, absence of cleavage; 5th, fusion of cells; 6th, unusual migrations of cells; 7th, death of cells. The presence of one or more of the above factors and their various combinations gives rise to the numerous monstrosities found during the cleavage, gastrulation and larval life; moreover one abnormality gives origin to others later on in development, so that larvæ with great defects are classed as cases of death, for instance, of one or more cells in an early stage.

In addition to the various abnormalties thus classified some other forms, such as a larva with well-developed double or bifid tail, were observed but not traced back to any of the above seven categories.

The interest of these various modes of irregularity lies, for our present purposes, in the fact that all seven conditions have been artificially brought about by M. Chabry by various mechanical stimuli applied either to the egg of the ascidian or to that of the sea urchin. The results upon the ascidian were for the most part obtained by means of traumatic inter-

ference, and lead only to the death of cells. These abnormalities are the ones described in the sequel, while other classes of abnormalities are either obtained by other methods applied to the egg of the same animal or else refer to the egg of the sea urchin, *Strongylocentrotus lividus*, and are not mentioned in detail in the present paper.

Wounding the cells of *Ascidia aspera* in early stages leads to the death of these cells and to subsequent abnormalities of development identical with those resulting when the cells die naturally or without apparent cause.

The method of inflicting injuries upon one or more cells of the minute eggs studied by M. Chabry is as simple in principle as it is successful in operation, given sufficient delicacy in manipulation. The eggs are observed under the microscope in capillary tubes of glass, each egg lying without undue pressure in a separate tube of right diameter. The tube is mounted in water and covered by a cover glass so that a clear view of the egg is obtained with quite high powers. To see all sides of the egg the tube is revolved by a small crank and wheel attached to one end, turning freely in two rings of glass fixed to the microscopic slide.

The other end of the tube bears the exceedingly sharp pointed needle that is to perforate the egg. This needle is the most difficult part of the apparatus to manufacture, being a glass rod drawn out to a point of excessive acuteness and also straight. When once made the needles are provided with a protecting piece of capillary tubule, which may either pass into the capillary containing the egg or else be joined to its end by a surrounding tube according as the egg capillary is large or very small. To move the glass dart in and out, towards and away from the egg when it has been first rightly adjusted, a small lever attached to the microscope by an ingenious and simple arrangement of spring and screw enables one to thrust the point, while observing it under the microscope, into the egg for a given distance and not further, and then to withdraw it quickly. Thus stabs are made that need affect but a single cell and any known and chosen cell.

Such is the accuracy and delicacy of this apparatus that the sea urchin's egg, only one tenth of a millimeter in diameter, was actually pierced by the finer glass needles.

To begin with this latter experiment upon the sea urchin, the needle is followed by sea water, which remains in part within the egg when the needle is withdrawn but yet gradually disappears as the egg closes in over the wound and does not afterwards exercise any evil influence upon the subsequent development of the egg. An egg entirely pierced from one side to the other subsequently formed a normal pluteus.

In the ascidian, however, the perforation of the egg or of one of its cells gives rise to its death. Within a minute after the stab is made in the protoplasm of the cell an appearance of opacity or turbidity is seen rapidly extending through the entire cell; the cell dies. Later the protoplasm coagulates and remains fixed in whatever form it was fashioned by the pressure of adjacent cells when it died. This death of the cell by stabbing is a different, much more rapid, process from the gradual death observed to occur in many eggs of abnormal origin. The part of the egg not injured develops, however, just as when the death of its fellow cell was natural or of unknown origin. In this development certain definite rearrangements of the cells take place, since they are no longer held in normal positions by the attraction of the dead cell; then cleavage continues and finally imperfect larvæ result.

Some examples of the results obtained may be given here in detail. In normal cleavage the egg divides first into a right and a left cell, these divide into anterior and posterior cells, and the four resulting cells then divide equatorially into oral and aboral cells. If, in the two-celled stage the left one be killed, the right divides into an anterior and a posterior cell as if nothing had happened to the egg, and then these two divide as usual but arrange themselves much as if the dead cell were not present, pressing together into a spheroidal mass so that the posterior upper and anterior lower cells come into contact diagonally on one side next the dead cell or median plane, just as do the other two cells on the outside or right of the egg. Thus under the influence of

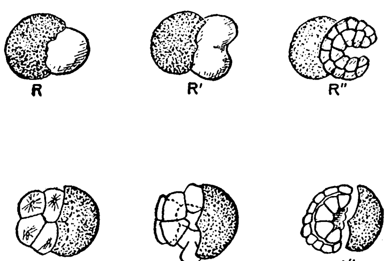
mutual attraction the four cells move so that they are arranged in a tetrahedron rather than in a square. The next change is a division of each by a plane parallel to the median plane or to the face of the dead cell.

This illustrates a marked tendency in all natural cases of death; that is, the planes that normally would be nearly meridional, turn so as to become parallel to the dead cell, parallel to a plane passing through the center of the egg.

From this eight-celled stage the development proceeds till a larva is formed, having a normal tail, three distinct germ layers, a pigmental area representing the nervous system and one papilla for attachment. Having begun to secrete its cellulose mantle it died.

Other cases were obtained showing the same results. Figure 2, R, R′, R″ shows three successive stages in the development of one of these artificial right-half embryos compared with successive stages, L, L′, L″, in the development of a left-half embryo of natural or unknown origin.

FIGURE 2.



Again, when the posterior left of the normal four cells, after two planes have occurred in cleavage, is killed by the needle, an ovoid larva is obtained. In this the tail adheres along the trunk and there are three papillæ of attachment and two pigment spots. Similar monstrous forms are found when the right posterior cell is killed. When the right anterior cell is killed the tail is well formed and free from fusion with the trunk, and there are no pigment spots or imperfect ones. When the left anterior cell is killed the larva has a perfect tail, a papilla for fixation, a pigment spot and active movement. It escaped from most of the egg membranes and secreted a tunic, into which migratory cells were passing when it died.

Killing two diagonal cells of four is followed by a normal cleavage of the two remaining cells, but the experiment was

interrupted here. Killing both left cells of the four, results in the formation of larvæ which are imperfect in that there is but one papilla for attachment and one atrial invagination with one pigment spot or eye. Similar monsters result from killing both right cells, but the eye spot is absent.

When three of the four cells are killed the remaining one divides normally and forms a rounded mass of cells arranged in two germ layers, but the development does not continue further. Likewise by thrusting the needle amongst the cells of a cleaving egg, though some are killed a few may be separated and then live isolated in the sea water. Such a cell divides, with karyokinesis, into two, four, eight cells by planes at right angles, then the normal rearrangement and adjustment of the cells take place as if an entire egg were in question. The cleavage planes also occur at intervals of 20 minutes as in the entire normal egg. There results after some hours a rounded mass of twenty or so cells, larger than the original one, but there the development ceases.

In such experiments M. Chabry sees a new method of anatomical research; the history of each cell may be followed from early to late stages by killing it and observing the consequent lack in the resulting imperfect later stages. Though it is unsafe to conclude from the disappearance of an organ after the death of some particular cell at an earlier stage that that cell would have formed the organ, yet by killing all the other cells, one by one, and finding the organ present in all the resulting stages, its dependence in the normal condition upon the cell first killed becomes conclusive. In this way the author traces the eye to the right anterior cell of the first four of cleavage, the otolith to the right posterior cell; the two papillæ for attachment come from the two anterior cells while the chorda is formed by both anterior and posterior cells. Nevertheless as left-half larvæ are sometimes found with an eye, and other such cases occur, the above is upheld by the author only by aid of the supposition that the surviving cells change their habit after the death of the one, so that they now produce organs they would not normally. Thus the eyes are potentially two, though but the right one is normally produced.

Similarly the notochord is to be regarded as double or composed of two halves, one in each of the first two cells.

Nevertheless, M. Chabry regards the egg of *Ascidia aspera* as containing potentially but one adult, the organs of which seem to be localized in different parts of the egg. That this is necessarily true of other eggs he emphatically denies; the results here obtained cannot, he thinks, be extended to other untried cases. Granting that there is this localization of some organs in the ascidian egg it is evident from the author's account that all the structures are not divided by the first cleavage but rather that each cell has for the main part all that the other has, hence result active larvæ from either right or left cell, if the other be killed, larvæ which are deficient in only a few organs and by no means real half-larvæ as the author calls them.

Finally we may consider some of the experimental work that has been recently attempted upon eggs of lower animals, the echinoderms especially.

Oscar and Richard Hertwig[1] subjected eggs of *Strongylocentrotus lividus* to the action of heat, poisons and mechanical insult, to judge from the effects upon external and internal fertilization and upon cleavage as to the nature of the forces involved in the normal course of events. All these unusual agents act upon the egg so that it is unable to keep out more than one sperm, and hence is penetrated by several or many sperm, exhibiting the abnormal phenomena of polyspermy.

Weak reagents cause only a few eggs to take in two or three sperms, while strong reagents cause most all the eggs to take in four or more sperms. The substances used and the strength as well as time are given in the following tables:

WEAK REAGENTS.

Nicotine	1 drop to 1000	for 10 minutes
Strychnine	.005%	for 20 minutes
Morphine	.1 to 2%	for 2 hours

[1]O. and R. Hertwig. Ueber den Befruchtungs- und Theilungs- vorgang des theirischen Eies unter dem Einfluss ausserer Agentien. Jen. Zeit. xx, 1887, pp. 120–227, 477–510, plates 3–9.

Cocaine	.025%	for 5 minutes
Quinine	.005%	for 5 minutes
Chloral	.2%	for 12 minutes
Heat	31°C.	for 10 minutes

STRONG REAGENTS.

Nicotine	.1%	for 20 minutes
Strychnine	.01%	for 20 minutes
Morphine	.4%	for 5 hours
Cocaine	.1%	for 5 minutes
Quinine	.005%	for 1 hour
Chloral	.2%	for 3 hours
Heat	31°C.	for 45 minutes

These do not all act alike; quinine, chloral and probably cocaine and overheating temporarily stops the movement of the sperm, a diminution in size of the fertilization elevations upon the egg, postpone cleavage one-half to one and one-half hours, interrupt cleavage of nucleus, sometimes making it take retrograde steps, and interfere with formation of rays within the protoplasm of the egg.

Nicotine and strychnine, on the other hand, seem to increase the activity of the sperm and the contractility of the egg protoplasm. Morphine appears to have an intermediate action.

The authors explain the occurrence of polyspermy after the action of these agents as being due to the lack of normal sensitiveness on the part of the ovum. For normally the entrance of one sperm causes the egg to throw off a membrane which prevents the entrance of others, a membrane which is seen to be formed even upon fragments of eggs shaken loose before cleavage, yet when acted upon by these drugs the protoplasm of the egg is not sufficiently stimulated to form a membrane until several sperms have entered.

The internal fertilization, fusion of male and female nuclei, may be also retarded as much as one hour, and various unusual phenomena introduced in connection with the supernumerary sperms, without necessarily preventing the ultimate development of the oosperm.

Omitting many interesting facts bearing upon the value of male and female nucleus and cell protoplasm we pass to the effects upon the cleavage processes. The results are similar, whether the drugs act to produce polyspermy or whether they are subsequently applied after normal fertilization. Quinine or chloral acting upon an egg having its cleavage nucleus in the spindle stage transforms this spindle into a cluster of vesicles, but if the egg is now allowed to recover in sea water the nucleus divides into four with the formation of four combined spindles. The protoplasm, however, remains affected, and does not follow the subsequent division of each of the four nuclei.

The above work was supplemented soon after by a paper by Oscar Hertwig[1] describing the effects of cold upon the fertilization of the eggs of the sea urchin, and also recording the occurrence of abnormal eggs in most of the specimens found at Triest in the Spring of 1887; this result being apparently due to the unusual cold which prevented the animals collecting as usual when ripe (he finds the females discharge ova in the aquarium when a male has discharged sperm), and hence led to an overripe condition of the eggs, accompanied by subsequent abnormalities in development.

Eggs, he finds, may be kept for several hours at a temperature of 2° to 3°C. and yet recover, but they finally enter into a cold rigor. The cooling prevents the egg from forming its protective membrane and diminishes the receptive elevations upon the egg, and thus polyspermy results if the rigor does not intervene before the sperms have entered. Cooling after external fertilization may arrest the progress of the sperm, which is yet able to advance again when warmed. Cooling during the cleavage affects the nucleus so that various abnormal changes result, but the egg may still divide regularly when warmed, at least in a few cases.

One other suggestive experiment was made, namely, the treatment of sea urchins' eggs with methyl blue. This acts like a poison in causing polyspermy, yet weak solutions may be

[1]Oscar Hertwig. Experimentelle Studien am Tierischen Ei während, und nach der Befruchtung. Jen. Zeit., xxiv, 1890, pp. 268–310, plates 8–10.

used to stain the egg a violet color and yet not prevent it developing into a blastula in which the central fluid, the migratory cells and the inner ends of the outer cells are violet.

The method of inflicting mechanical injury upon sea urchin eggs used by the Hertwig's resulted in breaking them in some cases; this means of separation has been ingeniously put in action by Boveri in the attempt to solve a most important problem. Though the perusal of this paper[1] does not inspire one with as much confidence in the strength of the conclusions drawn as the reader would wish to have in evidence advanced in so important a case, yet the experiments are in themselves very suggestive and worthy of frequent repetition.

To prove that the nucleus is the bearer of inherited characters we may try to combine a nucleus with a cell and see which or if both transmit their peculiarities.

Using Hertwig's method he shook eggs of the sea urchin in test tube till many lost the nucleus; these could be fertilized and developed. In this way dwarf larvæ, about one-fourth the normal size, were reared as late as the seventh day, when the normal larvæ died also. Now the great interest of these experiments for the present question lies in the fact that the eggs belong to one species and the sperm to another.

When true bastards between normal eggs of *Echinus microtuberculatus* and sperm of *Sphærechinus granularis* are formed the resulting larva has always a middle form between the larvæ of these species, both in general proportions and in arrangement of skeletal spicules. When, however, broken fragments of the eggs of the first species are fertilized by sperm of the second we find beside some true bastards from the normal eggs and some small ones from nucleated fragments, some larvæ exactly like those reared from pure eggs and sperm of the second species alone, *Echinus microtuberculatus.*

These larvæ are regarded by Boveri as due to the fertilization of denucleated eggs of the other species by the sperm of the species they resemble.

[1]Boveri. Ein geschlechtlich erzeugter Organismus ohne mutterliche Eigenschaften. Sitzb. d. Gesellschaft f. Morphologie u. Physiologie in Munich, v, 1889, pp. 73–80.

The larvæ, when killed and examined, are found to have abnormally small nuclei, which is accounted for by the supposition that the single male nucleus of the sperm does not furnish as much material as the male and female nuclei normally do when combined.

If we accept these statements we have, indeed, most conclusive proof that in a male nucleus the sperm may transfer to a new organism the qualities of its parent.

The difficulties of the experiment lie in the unfavorable nature of the hybridization, only one in a thousand eggs being fertilized, so that of 200 actually isolated none happened to develop; then again the various abnormalities, half embryos and dwarfs that we may assume occur, make it a difficult question, we think, to decide as to the specific characters of the larvæ being due to inheritance or accidental resemblance from imperfect development.

The recent work of Driesch[1] is the last contribution in experimental embryology that has come to our notice. To determine the effect of light upon cleaving eggs he exposed the eggs of *Echinus microtuberculatus, Planorbis carinatus* and *Rana esculenta* to daylight and to complete darkness as well as to variously colored light. The result was that not only cleavage but also the formation of organs took place quite normally in time and form, entirely irrespective of the presence or character of light.

The more noticeable and unexpected part of the paper, however, deals with the question of self differentiation, as illustrated by experiments upon sea urchin eggs (as yet he has not succeeded in applying appropriate methods to eggs of frogs and Planorbis).

The eggs of the sea urchin in the two celled stage are shaken vigorously for five minutes in a test tube (some may need repeated shaking), and then such isolated cells as are present are quickly picked out and examined under the microscope in separate dishes of sea water.

[1]Hans Driesch. Entwicklungsmechanische Studien. I. Der Werth der beiden ersten Furchungs-zellen in der Echinodermenentwicklung. II. Über die Beziehung des Lichtes zur ersten Etage der theirischen Formenbildung. Zeit. f. wiss. Zool., liii, 1891, pp. 160–183, plate 7.

In this way as many as fifty cells were isolated from the two celled stages and kept separate in small vessels in which they could be examined microscopically and actually seen to develop. The separated cells develop at first, as do the uninjured cells of the frog in Roux's experiments, but the ultimate result is that each cell (of the two after the first cleavage of egg) may give rise to a complete though small larva.

This is found to be the case in both Echinus and Sphærechinus.

In the normal cleavage of Echinus, according to Selenka, there are formed two, four, eight cells, and then four at one pole bud off four small cells, while the four at the other pole divide equally. Now in the cleavage of the half egg Driesch finds two and then four cells, followed by an eight celled stage which is formed by or budded from two little cells at one pole, as opposed to an equal division of the two cells at the other pole; thus the eight celled stage is exactly the half, in form and arrangement, of the normal sixteen celled stage.

These half formations, however, become over night converted into complete blastulas having half the normal size, but apparently made up of cells of normal dimensions, so that we may infer there is half the normal number of cells. About thirty dwarf blastulas were obtained from isolated cells, and from these normally formed gastrulas, and eventually, in three cases normally formed dwarf plutei were reared. Thus it would be possible to obtain two plutei from one egg by separating the first two cells of cleavage.

The application of this to the explanation of the occurrence of twins is made easier by the actual finding of numerous abnormal stages in cleavage and gastrulation resulting in the production of twin gastrulæ or larvæ, or in some cases of combination of three-fourths and one-fourth blastulæ. As these occur so frequently in material submitted to the shaking process, which variously effect different eggs, we have reason to suppose the twins are directly due to the mechanical separation or disturbance of the material in the egg.

The heterogeneous character of the various experiments referred to in the present article and the conflicting and often

apparently meaningless results obtained by one or the other of the experimenters, while preventing an immediate incorporation of the facts of experimental physiology with those recorded by the purely observational school should not blind us to the importance of the work thus far done, both as a good beginning in a promising field and as already furnishing valuable controls for the guidance of speculations upon some of the most fundamental questions in biology.

"Toutes les expériences, toutes les mutilations qui èn fait subir à un oeuf normal, contribuent, en effet, à devoiler sa structure, et c'est certainement là une des plus belles recherches que le naturaliste puisse se proposer."